PROTOCOL

A Study to Determine the Etiology of Southern Tick-Associated Rash Illness (STARI) in the United States

Background/justification

Lyme disease is due to infection with the tick-transmitted spirochete, *Borrelia burgdorferi*. In the United States, the regions with the highest incidences of Lyme disease are the Northeast, Upper Midwest, and northern Pacific Coast. Twelve states account for 95% of cases reported nationally (Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin).[1]

Erythema migrans (EM), the characteristic annular, macular, erythematous skin lesion of early Lyme disease, occurs at the site of the infected tick bite, has an incubation period of 3-31 days, and typically expands over time, sometimes to a diameter of $30 cm. *Borrelia burgdorferi* can be readily cultured from the skin or blood of patients with EM acquired in Lyme disease endemic areas[2], but this spirochete has not been isolated from persons suspected to have Lyme disease acquired in the southern and south-central United States to date.[3-6]

Tick bite-associated annular, expanding, erythematous, EM-like lesions do occur in patients in the southern and south-central United States. The etiology, natural history, incidence, public health importance and appropriate treatment of Southern tick-associated rash illness (STARI) are unknown. Three systematic studies of patients with this condition have been published.[3-5] Many of these patients received at least one empirical course of antibiotics. Such cases are often reported as Lyme disease: 3,552 cases of Lyme disease were reported to CDC from southern states and Missouri during 1993-2002.[1]

Some EM-like lesions seen in the south appear to be associated with bites of *Amblyomma americanum* (the Lone Star tick), the most common human-biting tick in the region. Studies to date have failed to convincingly implicate *B. burgdorferi* or other known arthropod-borne agents in these cases [unpublished data].[3-6] Possible etiologies of EM-like rashes in this region include a novel[7] and newly cultivated[8] spirochete seen in 2-4% of field-collected *A. americanum* [7,9] and described by molecular techniques as *Borrelia lonestari* sp. nov.,[7] or another infectious or noninfectious agent.

We have recently described a patient with EM and an attached lonestar tick, implicating *B. lonestari* as a pathogen. *B. lonestari* DNA sequences were amplified from a skin biopsy sample donated by the patient and from the attached tick.[10]

It is important to understand the etiology of Lyme disease-like illness in the southern United States for both public health and clinical reasons. The case definition for surveillance of Lyme disease, a nationally notifiable condition, counts all persons with EM as cases. Counting STARI as Lyme disease affects both the accuracy and the credibility of surveillance statistics for Lyme disease. The Lyme disease bacterium is generally absent in ticks and their natural hosts in southern states, and the preponderance of evidence indicates that people are not at risk of Lyme disease in this area. Nevertheless, thousands of people have been reported as having Lyme disease in the south because they have EM. Furthermore, clinicians do not have a rational basis for decisions about the treatment of STARI patients, or even know if prompt antibiotic therapy is necessary. Whereas appropriate treatment of early Lyme disease is essential to minimize serious sequelae (arthritis, profound fatigue, pain, or cognitive

impairment), STARI may be a simple, self-limiting skin rash. However, the natural history of STARI is inadequately understood, and the possibility of chronic debilitation cannot be excluded.

Laboratory diagnostic tests specific for STARI could in principle be developed if the etiologic agent could be determined. Besides the obvious value to doctors and their patients, a laboratory test for STARI could clarify the performance of diagnostic tests and candidate vaccines for Lyme disease. EM patients in the south are almost always seronegative for antibodies to *B. burgdorferi,* even at convalescence. The combination of thousands of Lyme disease cases reported and lack of positive Lyme disease antibody tests bolsters the claim that serologic tests for Lyme disease are poor. Similarly, clinical trials of a first generation Lyme disease vaccine, now removed from the market, documented break-through EM in some vaccinees. When, or if, a second-generation vaccine for Lyme disease enters clinical trials, it will be important to be able to distinguish among the various causes of EM.

Objectives

1) To evaluate the hypothesis that *B. lonestari* is the cause of STARI in the United States. To meet this objective, we wish to obtain skin biopsy specimens, whole blood, and acute and convalescent serum samples from patients with tick bite-associated, expanding, erythematous, rash lesions. Skin samples will be tested by polymerase chain reactions (PCR) targeting the *glpQ*[11] and 16S rRNA [J. Bunikis, unpublished] genes and by culture, when available. Serum will be used to develop serologic tests for *B. lonestari* infection using recombinant GlpQ and antigens isolated from co-culture of this spirochete with tick cells.
2) To store portions of each specimen in an appropriate fashion to permit future testing of other etiologic hypotheses, once additional test methods are available.

Locale

Southern United States and adjoining states

Number of Subjects

Approximately 20 patients annually for each of three years.

INVESTIGATORS/COLLABORATORS

Principal Investigator:

Barbara J. B. Johnson, PhD
Chief, Microbiology and Pathogenesis Laboratory
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Dr. Johnson will have primary responsibility for this protocol, co-primary responsibility for recruitment of patients, and responsibility for the design of the specimen collection kit, testing of specimens by currently available methods, and development of new tests, particularly serology to detect antibodies to *B. lonestari.*

Co-Investigators:

Paul S. Mead, MD, MPH
Chief, Epidemiology, Microbiology, and Diagnosis Activity
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Jacob Kool, MD, PhD
Epidemiologist, Epidemiology, Microbiology, and Diagnosis Activity
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Drs. Mead and Kool will have co-primary responsibility for communicating with potential collaborating physicians, and for acquisition and storage of clinical data in accordance with requirements to protect human subjects.

Martin E. Schriefer, PhD
Chief, Diagnostic and Reference Laboratory
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Dr. Schriefer will be responsible for laboratory testing of samples for evidence of infection by Lyme disease bacteria.

Rendi M. Bacon, MS
Microbiologist, Microbiology and Pathogenesis Laboratory
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

LT Bacon will collaborate in the development of new diagnostic tests.

Steven L. Sviat, BS
Microbiologist, Microbiology and Pathogenesis Laboratory
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Mr. Sviat will be responsible for preparing kits for shipping to collaborating physicians and receiving and logging in specimens.

Mark Pilgard, BS
Regular Fellow, Microbiology and Pathogenesis Laboratory
Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases, NCID, CDC
Fort Collins, Colorado

Mr. Pilgard will isolate DNA and perform PCR on appropriate samples, especially skin.

METHODS & MATERIALS

Study design

Case series

Sampling scheme

Convenience sample

Data collection methods

The necessary forms and a specimen collection kit will be sent to physicians as they contact study investigators about possible study participants. The laboratory submission form will be completed by the study participant's personal physician or a study investigator.

Sample sizes

No sample size calculation indicated or performed.

Data analysis methods

Analysis depends on diagnostic tests available and conducted, some of which are yet to be developed. No complicated statistical analysis anticipated.

Plans for reporting results

If results are of sufficient interest, they will be prepared for publication in a peer-reviewed journal.

As participants are recruited, the state health department will be notified and summary results will be provided.

Clinical procedures

Standard methods will be used by the personal physician of a study participant to collect skin punch biopsy samples and blood (see "Physician Instructions for Collecting and Handling Clinical Specimens and Data from Patients with Suspected Tick Bite-Associated Rash Lesions of Unknown Etiology in the Southern United States"). For most patients, two 2-mm punch biopsies of the skin lesion will be done. One specimen will be placed in chilled transport medium (PBS, sterile phosphate-buffered saline). When there is a second skin specimen, it will be placed in Streck's tissue fixative, or formalin if Streck's fixative is not available.

Skin biopsy samples will not be taken from tissue of the face or neck. A wound care instruction sheet will be provided to each patient who consents to skin biopsy.

Blood and serum will be collected in the standard manner. For adults, a single tube of blood (about 10 mL in an EDTA tube, customarily with a lavender-colored top) and a single tube of serum (also about 10mL of blood in a serum separator

tube with a red/gray top) will be obtained at the time the EM rash is first observed by a health care professional. A second single tube of serum will be collected 3-6 weeks later. For minors, a volume of less than 10 mL per sample may be collected at the discretion of the collaborating physician.

Patients will be requested to make an appointment for collection of a convalescent serum sample on the day that the acute-phase sample is collected. The office of the collaborating health care provider will call patients at home, if they miss the appointment for collection of the convalescent-phase sample, to reschedule it. Serum antibodies to the agent of Lyme disease reach maximum titers several weeks after antibiotic treatment for EM. Lacking knowledge of the time course of antibody development to the etiologic agent of STARI, a 3-6 week time to collection of a convalescent sample was selected by analogy with Lyme disease.

Laboratory techniques

Laboratory testing will focus initially on evaluating samples for infection by *B. lonestari* sp. nov. TaqMan PCR will be performed on DNA extracted from skin samples. When procedures developed by Varela *et al.*[8] are established in our laboratory, a portion of the unfixed skin samples and fractions of fresh whole blood (e.g., buffy coat) will be cultured to attempt to isolate *B. lonestari* from patients. Fixed skin samples will be stored for eventual immunohistochemical evalution (and comparison with fixed skin samples from cultured-confirmed *B. burgdorferi* infection that are not part of this protocol). Serum samples will be tested for antibodies to *B. burgdorferi*. The majority of the serum volume will be stored until a serologic test for antibodies to *B. lonestari* is developed.

If requested by individual physicians on a CDC DASH form, standard serologic test results for Lyme disease will be reported.

If evidence of *B. lonestari* infection of skin or blood samples is not obtained, remaining portions of samples will be stored in a confidential manner during this study. Alternative testing approaches may be pursued to identify the etiologic agent, but only after these testing methods have been described to the CDC IRB as a proposed amendment to this protocol, and have been approved for study.

Long term storage of samples

At the conclusion of this study, remaining portions of samples will be saved under code for possible future testing to determine the etiology of STARI. Patient records will continue to be locked to assure confidentiality. If new testing methods become available, a new protocol will be submitted to the CDC IRB before any laboratory work is performed with the stored specimens.

No genetic analysis will be performed on human DNA. No testing for HIV/AIDS will be performed.

Products

None anticipated

FDA, IND, or IDE information

None

PARTICIPANTS

Procedures for identifying/selecting

Since 1991, numerous individual physicians in the South have been in contact with CDC staff concerning the differential diagnosis and management of EM-like skin lesions in their patients. Many of these physicians are aware of CDC's interest in patients with these lesions and have stated their intention to contact CDC staff when additional such cases come to their attention. These physicians will be provided with specimen collection kits and forms at the beginning of the study. State epidemiologists in the appropriate states are also aware of Lyme disease-like illnesses in patients who reside in the South. They have been a source of contacts with physicians who are seeing patients who meet the case definition for this study. Finally, patients regularly contact CDC directly after reading about STARI on the website of the Division of Vector-Borne Infectious Diseases.

The following case definition will be used to determine eligibility for enrollment:

A person who is at least three years old with acute onset (within 14 days of visit to a physician's office) of an annular, erythematous, expanding EM-like rash that attains a size of at least 5 cm in diameter, when no alternative explanation for the rash can be found, AND who has a history of tick bite at the rash site, or potential exposure to ticks within 14 days prior to rash onset AND who will consent to storage of biologic samples for later testing.

Exclusion criteria: Any person with hemophilia or other coagulopathies, including patients taking potent anticoagulants such as warfarin. Also, any immunocompromised patient or person currently undergoing chemotherapy.

Exclusion from skin biopsy portion of study:

A person who meets the case definition but whose EM-like rash occurs on the face or neck will not be enrolled for purposes of obtaining a skin biopsy specimen. Such a person may enroll for purposes of providing a clinical history and blood samples only.

Vulnerable populations

Children are often at higher risk for tick-borne diseases than are adults, because of increased exposure to tick-infested habitat and perhaps other factors. For this reason, we intend to enroll children who are at least three years old into our study, with parental permission.

Emergency care procedures for an adverse event

Via the informed consent form, patients will be instructed to contact their personal physician in the event of surgical (punch biopsy) wound complications (e.g., redness, exudate, or hemorrhage).

Procedures for notifying participants of their individual results and study results

Participants will be notified of the result of testing for antibodies to *B. burgdorferi* via their personal physicians, which physicians request such testing. Test results for evidence of agents other than *B. burgdorferi* usually will not be available in a time frame that will be useful to the care of patients. Communication of such test results, therefore, will not be part of this protocol.

RISK/BENEFIT INFORMATION

Potential risks, likelihood and seriousness

Complications of phlebotomy are unlikely, although local infection and small to medium-sized hematomas may occur.

The most common anticipated complications are infections or minor hemorrhage of the skin biopsy site(s). Since most, if not all, STARI patients are likely to be prescribed empirical courses of broad-spectrum antimicrobials (most commonly tetracycline, doxycycline, or amoxicillin--- the standard of care for early Lyme disease) for their rash illness,[4,5] surgical (punch biopsy) wound infections should be even more rare than usual and readily managed with local heat and, rarely, culture and an appropriate change of antimicrobials. Biopsy-site bleeding should be mild and readily manageable with local pressure. When healed, 2-mm skin punch biopsies leave a tiny (~1-mm-diameter), permanent scar of little cosmetic impact, especially if non-facial in location.

Methods to minimize risks

To minimize the risk of biopsy site infection, biopsy sites will be disinfected in the standard fashion for minor surgical procedures. Hemostasis will be achieved with pressure and, optionally, application of a butterfly bandage or single suture per 2-mm biopsy site. (Two-mm punch biopsy sites rarely require suturing for hemostasis). To minimize the risk of bleeding complications, persons with hemophilia or other coagulopathies, and persons taking potent anticoagulants (e.g., warfarin), will be excluded. Taking nonsteroidal anti-inflammatory drugs alone will be not be considered a sufficient criterion for exclusion. To minimize the impact of scarring, no facial skin lesions will be biopsied.

To minimize the risk of phlebitis, phlebotomy sites will be disinfected with a tincture of iodine followed by an alcohol swab. For further information, see "Physician Instructions for Collecting and Handling Clinical Specimens and Data from Patients with Suspected Tick Bite-Associated Rash Lesions of Unknown Etiology in the Southern United States" (attached).

Benefits

There will be no direct benefit to study participants. Indirect benefit to individual participants will be in the form of a benefit to the community at large, through helping public health officials to understand the cause, natural history, public health importance, and optimal treatment of STARI.

INFORMED CONSENT PROCEDURES

Procedures to be used

A copy of the *Belmont Report: ethical principles and guidelines for the protection of human subjects of research* will be sent to collaborating physicians by facsimile or the website link http://ohsr.od.nih.gov/guidelines/belmont.html will be sent by e-mail. After the physician has reviewed the Belmont Report, he or she will be asked to sign an Unaffiliated Investigator Agreement (UIA) to be sent to CDC. Samples will not be accepted unless a UIA is signed and submitted.

Informed consent forms also will be sent by facsimile or e-mail to the patient's physician. The patient will sign two copies of these forms at the physician's office. One copy of the signed forms will be sent back to CDC with the clinical specimens; the other copy will be retained by the patient. For minors or wards, an assent form for minors will be obtained in addition to the parent or guardian signing an informed consent on behalf of that child or ward.

Requests/justifications for any waivers

None.

Indication of whether forms/statements were approved by legal advisor

No.

RECORDS MANAGEMENT

Description of forms to be used

1) Unaffiliated Investigator Agreement
2) "Physician Instructions for Collecting and Handling Clinical Specimens and Data from Patients with Suspected Tick Bite-Associated Rash Lesions of Unknown Etiology in the Southern United States"
3) "Laboratory Submission form for Southern Tick-Associated Rash Illness (STARI) Specimens"
4) Adult Consent/ Parental Permission/ Adolescent Assent Form
5) Child's Assent Form
6) CDC DASH form to request serologic tests for Lyme disease (optional).

Methods to protect confidentiality

Patient names will be collected on both the Adult Consent/ Parental Permission/ Adolescent Assent Form and Child's Assent Form. After being completed, these forms will be returned to CDC investigators and kept confidential to the extent legally possible. Consent forms will be locked in the office of Dr. Paul Mead. Laboratory submission forms under the STARI protocol will lack personal identifiers. Any DASH forms submitted will be kept separate and filed with

patient identification protected as is currently done for routine diagnostics at CDC/DVBID. Dr. Martin Schriefer is responsible for routine diagnostics under CLIA-regulated protocols.

Justification for collection of sensitive information
No sensitive information will be collected.

Applicability of Privacy Act
Not applicable

ASSURANCES OF CONFIDENTIALITY

Formal assurance of confidentiality obtained/planned
None/none

Certificate of confidentiality obtained/planned
None/none

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